

What is claimed is:

- 1 1. A method for detecting a GPCR-binding partner complex comprising a GPCR and a GPCR
2 binding partner, said method comprising:
3 (a) culturing a cell producing a first and a second polypeptide, wherein at least one of said first
4 and said second polypeptides is a GPCR,
5 (b) lysing said cell;
6 (c) contacting said first polypeptide with a substrate having affinity for said first polypeptide,
7 under conditions suitable for binding of said first polypeptide to said substrate; and
8 (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting
9 is direct;
10 wherein the presence of said second polypeptide on said substrate is indicative of a GPCR-binding
11 partner complex.
- 1 2. The method of claim 1, wherein said first polypeptide is a GPCR and said second polypeptide
2 is a GPCR binding partner.
- 1 3. The method of claim 1, wherein said first polypeptide is a GPCR binding partner and said
2 second polypeptide is a GPCR.
- 1 4. The method of claim 1, wherein at least one of said first and said second polypeptides is an
2 orphan GPCR.
- 1 5. The method of claim 1, wherein at least one of said first and second polypeptides is a native
2 GPCR.
- 1 6. The method of claim 1, wherein at least one of said first and said second polypeptides is a
2 constitutively activated GPCR.
- 1 7. The method of claim 1, wherein both of said first and said second polypeptides are GPCRs.
- 1 8. The method of claim 7, wherein said GPCRs are the same GPCR.
- 1 9. The method of claim 7, wherein said GPCRs are different GPCRs.

- 1 10. The method of claim 1, wherein one of said first and said second polypeptides is not a GPCR.
- 1 11. The method of claim 10, wherein said one of said first and said second polypeptide is a G-
2 protein.
- 1 12. The method of claim 1, wherein at least one of said first and said second polypeptides is
2 recombinant.
- 1 13. The method of claim 1, wherein said first polypeptide is bound to said substrate using an
2 antibody that specifically binds to said polypeptide.
- 1 14. The method of claim 1, wherein said first and second polypeptides are different and wherein
2 detection of said second polypeptide comprises binding of an antibody specific for said second
3 polypeptide.
- 1 15. The method of claim 1, wherein said first polypeptide comprises an affinity tag, and said
2 isolating step comprises binding of the affinity tag to the affinity substrate.
- 1 16. The method of claim 15 wherein said affinity tag is an epitope tag.
- 1 17. The method of claim 1, wherein said second polypeptide is detectably labeled, and said
2 detecting step comprises detecting said detectable label.
- 1 18. The method of claim 17, wherein said label is an epitope tag and said detecting comprises
2 binding of a fluorochrome-conjugated antibody specific for said epitope tag.
- 1 19. The method of claim 17, wherein said polypeptide is fused to a reporter protein.
- 1 20. The method of claim 19, wherein said reporter protein is luciferase.
- 1 21. The method of claim 19, wherein said first polypeptide is detectably labeled, and said detecting
2 step is by FRET.

1 22. The method of claim 1, wherein said method further comprises contacting said cell with a
2 cross-linking agent prior to said lysing step (b).

1 23. The method of claim 1, wherein said first and said second polypeptides are endogenously co-
2 expressed in at least one cell type, tissue, or tissue sub-region.

1 24. The method of any one of claims 1-22, wherein said method further comprises selecting prior
2 to said culturing step (a) said first and said second polypeptides wherein said first and said second
3 polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1 25. A method for detecting a GPCR-binding partner complex comprising a GPCR and a GPCR
2 binding partner, said method comprising:

3 (a) culturing a cell producing a first and a second polypeptide, wherein at least one of said first
4 and said second polypeptides is a GPCR,

5 (b) lysing said cell;

6 (c) contacting said first polypeptide with an addressable substrate having affinity for said first
7 polypeptide, under conditions suitable for binding of said first polypeptide to said substrate in an
8 addressable manner; and

9 (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting
10 is direct;

11 wherein the presence of said second polypeptide on said substrate is indicative of a GPCR-binding
12 partner complex.

1 26. The method of claim 25, wherein said first polypeptide is a GPCR and said second polypeptide
2 is a GPCR binding partner.

1 27. The method of claim 25, wherein said first polypeptide is a GPCR binding partner and said
2 second polypeptide is a GPCR.

1 28. The method of claim 25, wherein at least one of said first and said second polypeptides is an
2 orphan GPCR.

1 29. The method of claim 25, wherein at least one of said first and said second polypeptides is a
2 native GPCR.

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- 1 30. The method of claim 25, wherein at least one of said first and said second polypeptides is a
2 constitutively activated GPCR.
- 1 31. The method of claim 25, wherein both of said first and said second polypeptides are GPCRs.
- 1 32. The method of claim 31, wherein said GPCRs are the same GPCR.
- 1 33. The method of claim 31, wherein said GPCRs are different GPCRs.
- 1 34. The method of claim 25, wherein one of said first and said second polypeptides is not a GPCR.
- 1 35. The method of claim 34, wherein said one of said first and said second polypeptide is a G-
2 protein.
- 1 36. The method of claim 25, wherein at least one of said first and said second polypeptides is
2 recombinant.
- 1 37. The method of claim 25, wherein said method further comprises contacting said cell with a
2 cross-linking agent prior to said lysing step (b).
- 1 38. The method according to any one of claims 25-37 wherein said addressable affinity substrate is
2 spatially addressable.
- 1 39. The method of claim 38 wherein said spatially addressable affinity substrate is a 96-well or
2 384-well format affinity substrate.
- 1 40. The method of claim 38, wherein said first polypeptide is bound to said substrate using an
2 antibody that specifically binds to said first polypeptide.
- 1 41. The method of claim 38, wherein said first and said second polypeptides are different and
2 wherein detection of said second polypeptide comprises binding of an antibody specific for said second
3 polypeptide.

- 1 42. The method of claim 38, wherein said first polypeptide comprises an affinity tag, and said
2 isolating step comprises binding of the affinity tag to the affinity substrate.
- 1 43. The method of claim 42 wherein said affinity tag is an epitope tag.
- 1 44. The method of claim 38, wherein said second polypeptide is detectably labeled, and said
2 detecting step comprises detecting said detectable label.
- 1 45. The method of claim 44, wherein said polypeptide is fused to a reporter protein.
- 1 46. The method of claim 45, wherein said reporter protein is luciferase.
- 1 47. The method of claim 45, wherein said first polypeptide is detectably labeled, and said detecting
2 step is by FRET.
- 1 48. The method according to any one of claims 25-37 wherein said addressable affinity substrate is
2 spectrophotometrically addressable.
- 1 49. The method of claim 48, wherein wherein said first polypeptide is bound to said substrate using
2 an antibody that specifically binds to said polypeptide.
- 1 50. The method of claim 48, wherein said second polypeptide comprises an epitope tag, and said
2 detecting step comprises detection of the epitope tag.
- 1 51. The method of claim 50 wherein said detection comprises binding of a fluorochrome-
2 conjugated antibody specific for the epitope tag.
- 1 52. The method of claim 38 or claim 48, wherein said first and said second polypeptides are
2 endogenously co-expressed in at least one cell type, tissue, or tissue sub-region.
- 1 53. The method of any one of claims 25-51, wherein said method further comprises selecting prior
2 to said culturing step (a) said first and said second polypeptides wherein said first and said second
3 polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1 54. A method of identifying whether a candidate polypeptide is a binding partner for a GPCR,
2 comprising the step of detecting a GPCR-binding partner complex comprising said candidate
3 polypeptide and said GPCR according to the method of any one of claims 1-53, wherein detection of
4 said complex is indicative of said candidate polypeptide being a binding partner of said GPCR.

1 55. A method for detecting a GPCR-binding partner complex, said method comprising:

2 (a) culturing a plurality of cells, each cell producing a first and a second polypeptide, wherein
3 at least one of said first and said second polypeptides is a GPCR, and wherein each cell produces a
4 different GPCR;

5 (b) lysing said cells;

6 (c) contacting said first polypeptide from each cell with an addressable substrate having
7 affinity for said first polypeptide, under conditions suitable for binding of said first polypeptide to said
8 substrate at an address specific for said cell; and

9 (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting
10 is direct;

11 wherein the presence of said second polypeptide at an address on said substrate is indicative of a
12 GPCR-binding partner complex comprising said first polypeptide and said second polypeptides
13 produced by the cell having said address.

1 56. The method of claim 55 wherein said plurality of cells is at least 2, at least 5, at least 10, at
2 least 15, at least 20, at least 25, at least 50, or at least 100 cells.

1 57. The method of claim 55, wherein said first and said second polypeptides are endogenously co-
2 expressed in at least one cell type, tissue, or tissue sub-region.

1 58. The method of claim 55, wherein said method further comprises selecting prior to said
2 culturing step (a) said first and said second polypeptides wherein said first and said second
3 polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1 59. A method for detecting a GPCR-binding partner complex, said method comprising:

2 (a) culturing a cell, said cell producing a first and a plurality of a second polypeptide, wherein
3 each of said plurality of said second polypeptide is different and wherein at least one of said first and
4 said plurality of said second polypeptide is a GPCR;

5 (b) lysing said cell;

6 (c) contacting said first polypeptide with a substrate having affinity for said first polypeptide,
7 under conditions suitable for binding of said first polypeptide to said substrate; and

8 (d) detecting the presence of said second polypeptide on said substrate, wherein said detecting
9 is direct;

10 wherein the presence of said second polypeptide on said substrate is indicative of at least one GPCR-
11 binding partner complex comprising said first polypeptide and said second polypeptide.

1 60. The method of claim 59 wherein said GPCR-binding partner complex is detected, further
2 comprising repeating steps (a) to (d) one or more times with subsets of said plurality of said second
3 polypeptide, said subsets encompassing said plurality, until a GPCR-binding partner complex is
4 detected from at least one cell producing a said first polypeptide and a single said second polypeptide.

1 61. The method of claim 59 wherein said plurality of said second polypeptides is at least 2, at least
2 5, at least 10, at least 15, at least 20, at least 25 said, at least 50, or at least 100 of second polypeptide.

1 62. The method of claim 59 wherein said producing of a first and a plurality of a second
2 polypeptide is transient.

1 63. The method of claim 59, wherein said first and second polypeptides are endogenously co-
2 expressed in at least one cell type, tissue, or tissue sub-region.

1 64. The method of claim 59, wherein said method further comprises selecting prior to said
2 culturing step (a) said first and said second polypeptides wherein said first and said second
3 polypeptides are coexpressed in a least one cell type, tissue or tissue sub-region.

1 65. The method of any one of claims 55-64, wherein said first polypeptide is a GPCR and said
2 second polypeptide is a GPCR binding partner.

1 66. The method of any one of claims 54-64, wherein said first polypeptide is a GPCR binding
2 partner and said second polypeptide is a GPCR.

1 67. The method of any one of claims 55-64, wherein at least one of said first and said second
2 polypeptides is an orphan GPCR.

- 1 68. The method of any one of claims 55-64, wherein at least one of said first and said second
2 polypeptide is a native GPCR.
- 1 69. The method of any one of claims 55-64, wherein at least one of said first and said second
2 polypeptides is a constitutively activated GPCR.
- 1 70. The method of any one of claims 55-64, wherein both of said first and said second
2 polypeptides are GPCRs.
- 1 71. The method of claim 70, wherein said GPCRs are the same GPCR.
- 1 72. The method of claim 70, wherein said GPCRs are different GPCRs.
- 1 73. The method of any one of claims 55-64, wherein one of said first and said second polypeptides
2 is not a GPCR.
- 1 74. The method of claim 73, wherein said one of said first and said second polypeptide is a G-
2 protein.
- 1 75. The method of any one of claims 55-64, wherein at least one of said first and second
2 polypeptides is recombinant.
- 1 76. The method of any one of claims 55-64, wherein said first polypeptide is bound to said
2 substrate using an antibody that specifically binds to said polypeptide.
- 1 77. The method of any one of claims 55-64, wherein said first and second polypeptides are
2 different and wherein detection of said second polypeptide comprises binding of antibody specific for
3 said second polypeptide.
- 1 78. The method of any one of claims 55-64, wherein said first polypeptide comprises an affinity
2 tag, and said isolating step comprises binding of the affinity tag to the affinity substrate.
- 1 79. The method of claim 78 wherein said affinity tag is an epitope tag.

- 1 80. The method of any one of claims 55-64, wherein said second polypeptide is detectably labeled,
2 and said detecting step comprises detecting said detectable label.
- 1 81. The method of claim 80, wherein said label is an epitope tag and said detecting comprises
2 binding of a fluorochrome-conjugated antibody specific for said epitope tag.
- 1 82. The method of claim 80, wherein said polypeptide is fused to a reporter protein.
- 1 83. The method of claim 82, wherein said reporter protein is luciferase.
- 1 84. The method of claim 82, wherein said first polypeptide is detectably labeled, and said detecting
2 step is by FRET.
- 1 85. The method of any one of claims 55-64, wherein said method further comprises contacting said
2 cell with a cross-linking agent prior to said lysing step (b).
- 1 86. A method for identifying whether a candidate agent modulates binding of a GPCR to a binding
2 partner for the GPCR, said method comprising the step of determining whether there is a difference in
3 the amount of a GPCR-binding partner complex comprising said GPCR and said binding partner
4 detected according to a method of any one of claims 1-52, wherein said difference is determined for
5 contacting or not contacting said candidate agent with said first and said second polypeptides prior to
6 said detecting step (d) of said method and wherein an alteration in said amount of said second
7 polypeptide detected on said affinity substrate is indicative of an agent that modulates binding of said
8 GPCR to said binding partner for the GPCR.
- 1 87. The method of claim 86, wherein said first and said second polypeptides are contacted with
2 said candidate agent during step (a), step (b) or step (c).
- 1 88. The method of claim 86, wherein said candidate agent is a small molecule, a peptide, a ligand
2 for said GPCR, or an antibody.
- 1 89. The method of claim 86, wherein said modulator reduces said binding of a GPCR to a binding
2 partner for the GPCR by more than about 10%, more than about 20%, more than about 30%, more than
3 about 40%, or more than about 50% as compared to said binding in the absence of said agent.

- 1 90. The method of claim 86, wherein said modulator increases said binding of a GPCR to a
2 binding partner for the GPCR by more than about 10%, more than about 25%, more than about 50%,
3 more than about 100%, more than about 200%, more than about 300%, more than about 400%, or more
4 than about 500.
- 1 91. The method according to any one of claims 1-90 wherein said first and said second
2 polypeptides are both mammalian.
- 1 92. The method according to claim 91 wherein said first and second polypeptides are both human.
- 1 93. A composition comprising an addressable affinity substrate, said addressable affinity substrate
2 comprising a plurality of addresses having affinity for different GPCRs.
- 1 94. The composition of claim 93 wherein said addressable affinity substrate is spatially
2 addressable.
- 1 95. The composition of claim 94 wherein said spatially addressable affinity substrate comprises at
2 least about 100, at least about 200, at least about 300, at least about 400, or at least about 500
3 addresses.
- 1 96. The composition of claim 93 wherein said addressable affinity substrate is
2 spectrophotometrically addressable.
- 1 97. The composition of claim 96 wherein said spectrophotometrically addressable affinity substrate
2 comprises at least about 2, at least about 5, at least about 10, at least about 15, at least about 20, or at
3 least about 25 addresses.
- 1 98. A composition comprising a cell, said cell producing a first and a second polypeptide, wherein
2 said first and said second polypeptides are a GPCR and a GPCR binding partner, and wherein said first
3 polypeptide comprises an affinity tag and said second polypeptide is fused to a reporter protein.
- 1 99. The composition of claim 98, wherein said first polypeptide is a GPCR and said second
2 polypeptide is a GPCR binding partner.

- 1 100. The composition of claim 98, wherein said first polypeptide is a GPCR binding partner and
2 said second polypeptide is a GPCR.
- 1 101. The composition of claim 98, wherein at least one of said first and said second polypeptides is
2 an orphan GPCR.
- 1 102. The composition of claim 98, wherein at least one of said first and said second polypeptides is
2 a native GPCR.
- 1 103. The composition of claim 98, wherein at least one of said first and said second polypeptides is
2 a constitutively activated GPCR.
- 1 104. The composition of claim 98, wherein both of said first and said second polypeptides are
2 GPCRs.
- 1 105. The composition of claim 104, wherein said GPCRs are the same GPCR.
- 1 106. The composition of claim 104, wherein said GPCRs are different GPCRs.
- 1 107. The composition of claim 98, wherein one of said first and said second polypeptides is not a
2 GPCR.
- 1 108. The composition of claim 107, wherein said polypeptide is a G-protein.
- 1 109. The composition of claim 98, wherein at least one of said first and second polypeptides is
2 recombinant.
- 1 110. The composition of claim 98 wherein said affinity tag is an epitope tag.
- 1 111. The composition of claim 98, wherein said reporter protein is luciferase.

1 112. The composition of claim 98, wherein said GPCR and said GPCR binding partner of said first
2 and said second polypeptides are endogenously co-expressed in at least one cell type, tissue, or tissue
3 sub-region.

1 113. The composition of any one of claims 98-111, wherein said GPCR and said GPCR binding
2 partner of said first and said second polypeptides are selected prior to said producing for co-expression
3 in a least one cell type, tissue or tissue sub-region.

1 114. The composition of any one of claims 98-113 wherein said first and said second polypeptides
2 are both mammalian.

1 115. The composition of claim 114 wherein said first and said second polypeptides are both human.

1 116. A composition comprising a library of GPCRs, wherein said library comprises a plurality of
2 pairs of isolated polynucleotide wherein both a first and a second said polynucleotide of said pair
3 comprises a first nucleotide sequence encoding the same GPCR, said encoded GPCR of one said pair
4 being different from said encoded GPCR of any other said pair, and wherein said first polynucleotide
5 of said pair further comprises a second nucleotide sequence encoding an in-frame affinity tag and said
6 second polynucleotide of said pair further comprises a third nucleotide sequence encoding an in-frame
7 reporter protein.

1 117. The composition of claim 116 wherein said affinity tag is an epitope tag and said reporter
2 protein is luciferase.

1 118. The composition of claim 116 wherein said library comprises at least about 50 said pairs, at
2 least about 100 said pairs, at least about 200 said pairs, at least about 300 said pairs, at least about 400
3 said pairs, or at least about 500 said pairs.

1 119. The composition of claim 116 wherein said encoded GPCR is native.

1 120. The composition of claim 116 wherein said encoded GPCR is mammalian.

1 121. The composition of claim 120 wherein said encoded GPCR is human.